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Full Length Research Paper

Anti-obesity and hypolipidemic effects of a functional formula containing *Prumus mume* in mice fed high-fat diet

Daozong Xia[1,2], Xiaoqin Wu[1], Qing Yang[2], Jinyan Gong[1] and Ying Zhang[1]*

[1]Department of Food Science and Nutrition, College of Biosystem Engineering and Food Science, Zhejiang University, Hangzhou, China.
[2]College of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou, China.

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The present study is designed to investigate the effect of an anti-obesity functional formula (PMC) composed of *Prumus mume* stem barks extract (PMSBE) and L-carnitine (2:1, w/w) on obesity and hyperlipidemia induced by high-fat diet in mice. Male ICR mice were fed a high-fat diet (HFD) with or without PMC (PMC-1 group: 450 mg/kg/day; PMC-2 group: 900 mg/kg/day) for 6 weeks to examine adipose tissue weight, serum lipid profile, and the pathological changes in liver tissue. The results showed that body weight gain was significantly lowered in PMC groups compared with the HFD group. PMC could improve lipid profile by lowering serum total cholesterol (Total-C), triacylglycerol (TG), low-density lipoprotein cholesterol (LDL-C) concentrations, and increasing high-density lipoprotein cholesterol (HDL-C) concentration as compared with the HFD group. PMC also improved the pathological changes in liver tissue, decreased the relative weights of epididymal and perirenal WAT. Our findings suggest that PMC has significantly anti-obesity and hypolipidemic effects.

Key words: *Prunus mume*, anti-obesity functional formula, L-carnitine, high-fat diet.

INTRODUCTION

Obesity is one of the greatest health threats of this century, which has an important impact on life style-related diseases such as coronary heart disease, dyslipidemia, glucose intolerance, diabetics, hypertension and some cancers (Hu et al., 2008). Several factors, including lack of exercise, sedentary lifestyles and the consumption of energy rich diets are contributory to the etiology of obesity (Ekanem et al., 2007). Despite the urgent need for safe and efficient therapeutics and the potential size of the market for anti-obesity drugs, the current status for the development of such drugs is still unsatisfactory (Shrestha et al., 2007). Some edible medicinal plants have been used as dietary supplements for body-weight management and control in many countries (Bagri et al., 2009; Lee et al., 2008).

Mei (*Prunus mume* Sieb. *et* Zucc.) is a deciduous tree of Rosaceae family, which originated in Southeastern China and grown widely (Jo et al., 2006; Ning et al., 2007). The stem bark of *P. mume* is used as herbal medicines in several Asia countries (Matsuda et al., 2003). The stem bark extract of *P. mume* showed 28.4% inhibition of NO production in LPS-activated macrophages at the concentration of 80 µg/mL of sample in culture media (Ryu et al., 2003). There is no more information on the bioactive components and modern pharmacological effects of *P. mume* stem bark.

L-Carnitine is a small, water-soluble, quaternary amine that plays an important role in lipid catabolism in mammals (Lee et al., 2006; Yang et al., 2006).

*Corresponding author. E-mail: sigpx@sina.com; zhang_ying61@vip.163.com. Tel (Fax): +86 571 86049803.

Abbreviations: PMSBE, *Prunus mume* stem bark extract; **PMC,** PMSBE combined with L-carnitine (2:1, w/w); **OR,** orlistat; **ND,** normal diet; **HFD,** high-fat diet; **LPS,** lipopolysaccharide; **Total-C,** serum total cholesterol; **TG,** serum total triacylglycerol; **LDL-C,** low-density lipoprotein cholesterol; **HDL-C,** high-density lipoprotein cholesterol; **GAE,** gallic acid equivalents; **RE,** rutin equivalents; **ND,** normal diet; **IC_{50},** half maximal inhibitory concentration.

Supplementation of L-carnitine and antioxidants could lower plasma lipid concentrations and serum glucose, insulin and leptin levels in rats with HFD-induced obesity (Kim et al., 2008). *P. mume* stem bark extract combined with L-carnitine may have the latent capacity to reduce body fat and serum lipids in obese mammals.

The objective of this study was to investigate the effect of an anti-obesity functional formula (PMC) composed of *P. mume* stem barks extract and L-carnitine on obesity and hyperlipidemia induced by high-fat diet in mice.

MATERIALS AND METHODS

Preparation of *P. mume* stem bark extract (PMSBE) and powdered anti-obesity functional formula (PMC)

The stem bark of *P. mume* were obtained from a farm in Xiaoshan district (Hangzhou, Zhejiang province), air-dried, ground and extracted three times with 30% ethanol at 80℃ for 3 h each, then extracts were filtered using a strainer and spray-dried.

The anti-obesity functional formula (PMC) was prepared according to the project with New Era Health Industry (Group) Co., Ltd., China. The dried powder of *P. mume* stem barks extract (PMSBE) was passed through 40-mesh sieves. L-Carnitine was purchased from Jiangyin Haida Fine Chemical Plant (China). PMC was composed of PMSBE and L-carnitine in the proportion of 2:1 (w/w).

Determining total phenolics and total flavonoids contents in PMSBE

The amount of phenolic compounds in PMSBE was determined by the modified Folin-Ciocalteu method (Jimoh et al., 2007), and was expressed as milligrams of gallic acid equivalents (GAE) per g of PMSBE.

The flavonoids content in PMSBE was measured using a colorimetric assay developed by Pourmorad et al. (2006), and was expressed as milligrams of rutin equivalents (RE) per g of PMSBE.

Animal studies

The experimental diets consisted of: normal diet (ND), purchased from Shanghai SLAC Laboratory Animal Co., Ltd.; high-fat diet (HFD: ND supplemented with 10% egg yolk power and 10% lard), processed by Zhejiang Academy of Medical Sciences.

Fifty 7-week-old male ICR mice were obtained from Shanghai SLAC Laboratory Animal Co., Ltd. Mice were acclimated to the experimental facility for 1 week before they were divided into five groups of ten and housed in polycarbonate cages in a room maintained at 23 ± 1℃ and 55 ± 5% relative humidity. The room was exposed to alternating 12-h periods of light and dark. Ten mice (normal group) had free access to standard pelleted chow (normal diet, ND; Shanghai, China), and simultaneously administrated with deionized water at a dose of 10 mL/kg/day (orally) for 6 weeks. The remaining 40 mice were fed with a high-fat diet (HFD: ND supplemented with 10% egg yolk power and 10% lard) and randomly divided into 4 groups of HFD, HFD+PMC-1, HFD+PMC-2 and HFD+OR (orlistat). The HFD group simultaneously administrated with deionized water at a dose of 10 mL/kg/day (orally) for 6 weeks as ND group. The HFD+PMC-1, HFD+PMC-2 and HFD+OR groups simultaneously administrated with PMC (450 mg/kg/day, orally), PMC (900 mg/kg/day, orally), and orlistat (50 mg/kg/day, orally) for 6 weeks, respectively. The energy level of the high-fat diet in the HFD was 435 kcal/100 g, whereas that of the ND in the normal group was 360 kcal/100 g. The animals were given food and distilled water *ad libitum* during the experimental period. Food consumption and body weight gain were measured every week.

At the end of the experimental period, mice were sacrificed following a 14-h fast and collecting blood samples. The serum was separated by centrifugation at 3,000 rpm for 15 min and stored at -86℃ in order to determine the serum lipid profile. Livers were removed with the animals under anesthesia and rinsed with cold physiological saline and weighted, immediately frozen in liquid nitrogen and stored at -86℃ for further analysis. The adipose tissues (epididymal WAT and perirenal WAT) were immediately weighed. This investigation involving mice was carried out in accordance with the University Animal Care and Use Committee guidelines at Zhejiang University.

Quantitation of serum biochemical parameters

The concentrations of serum Total-C, TG, and HDL-C were assayed enzymatically using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). LDL-C was calculated from triglyceride, total cholesterol, and HDL-C concentrations using the following Friedwald formula (Friedewald et al., 1972):

LDL-C = Total-C - HDL-C - (TG/2.2).

Hepatic morphology

Livers were removed from the mice and fixed in a buffer solution of 10% formalin. Fixed tissues were processed routinely for paraffin embedding, and 4 μm sections were prepared and dyed with hematoxylineosin; stained areas were viewed using an optical microscope at ×400.

Statistical analysis

The data for the mice were expressed as means ± S.E.M. All statistical analyses were performed using SPSS 13.0 statistical software. Significant differences among the treatment means were determined using analysis of variance (ANOVA) and Duncan's multiple range test. Results were considered to be statistically significant at *P* values less than 0.05.

RESULTS

Total phenolics and total flavonoids contents of PMSBE

The total phenolics content of PMSBE was 401.0 mg/g, and the total flavonoids content of PMSBE was 355.5 mg/g.

Body weight and food intake

As shown in Table 1, body weight gain of the HFD group mice were considerably higher than the other groups. The weight gain of PMC-1, PMC-2 and OR groups were normalized to the ND group. Feed intake of all experimental groups had no significantly different.

Table 1. Effects of PMC on body weights in mice fed high-fat diet.

Parameter	Body weights				
	ND	HFD	OR	PMC-1	PMC-2
Initial body weight (g)	22.73 ± 0.19	22.79 ± 0.21	22.07 ± 0.28	21.94 ± 0.39	22.41± 0.24
Final body weight (g)	35.53 ± 0.78 [ab]	38.17 ± 1.01 [a]	34.08 ± 1.24 [b]	34.40 ± 0.84 [b]	35.06 ± 0.98 [ab]
Body weight gain (g/day)	0.29 ± 0.02 [b]	0.35 ± 0.02 [a]	0.27 ± 0.03 [b]	0.28 ± 0.02 [b]	0.29 ± 0.03 [b]

Values are mean ± S.E.M.; *n* = 10 in each group.
[ab]Means in the same row not sharing a common superscript are significantly different (P < 0.05) between groups.
ND, Normal control group; HFD, high-fat diet group; OR, high-fat diet with orlistat group; PMC-1, high-fat diet with PMC (450 mg/kg/day) group; PMC-2, high-fat diet with PMC (900 mg/kg/day) group.

Table 2. Effects of PMC on liver and adipose tissue weights in mice fed high-fat diet.

Tissue	Weight ratio (mg/g of body weight)				
	ND	HFD	OR	PMC-1	PMC-2
Liver	31.87 ± 2.09	36.31 ± 1.70	31.30 ± 1.48	32.21 ± 1.39	33.82 ± 1.28
Adipose tissue					
Epididymal WAT	15.13 ± 1.54 [c]	30.35 ± 2.74 [a]	20.43 ± 1.98 [bc]	25.12 ± 2.03 [ab]	22.51 ± 1.73 [b]
Perirenal WAT	2.78 ± 0.36 [b]	8.89 ± 0.95 [a]	5.89 ± 0.76 [b]	7.30 ± 0.96 [ab]	5.51 ± 0.39 [b]
Total	17.92 ± 1.82 [c]	39.26 ± 3.49 [a]	26.33 ± 2.59 [b]	32.41 ± 2.91 [ab]	28.02 ± 1.99 [b]

Values are mean ± S.E.M.; *n* = 10 in each group.
[a - c]Means in the same row not sharing a common superscript are significantly different (P < 0.05) between groups.
See Table 1 for a description of the different experimental groups.

Table 3. Effects of PMC on lipids concentrations in serum.

Lipid	Lipid levels in different groups				
	ND	HFD	OR	PMC-1	PMC-2
Total-C (mmol/L)	2.81 ± 0.08 [c]	4.75 ± 0.23 [a]	4.14 ± 0.14 [b]	4.04 ± 0.17 [b]	3.84 ± 0.14 [b]
TG (mmol/L)	1.13 ± 0.11 [b]	1.50 ± 0.08 [a]	1.00 ± 0.06 [b]	1.24 ± 0.07 [b]	1.14 ± 0.07 [b]
HDL-C (mmol/L)	1.09 ± 0.02 [a]	0.94 ± 0.02 [c]	1.03 ± 0.02 [b]	1.04 ± 0.02 [ab]	1.05 ± 0.02 [ab]
LDL-C (mmol/L)	1.50 ± 0.08 [c]	3.51 ± 0.22 [a]	2.91 ± 0.14 [b]	2.76 ± 0.17 [b]	2.56 ± 0.13 [b]
HDL-C/ Total-C	0.39 ± 0.01 [a]	0.20 ± 0.01 [c]	0.25 ± 0.01 [b]	0.26 ± 0.01 [b]	0.27 ± 0.01 [b]
LDL-C/ Total-C	0.53 ± 0.01 [c]	0.74 ± 0.01 [a]	0.70 ± 0.01 [ab]	0.68 ± 0.01 [b]	0.66 ± 0.01 [b]

Values are mean ± S.E.M.; *n* = 10 in each group.
[a-c]Means in the same row not sharing a common superscript are significantly different (P < 0.05) between groups.
See Table 1 for a description of the different experimental groups.

Liver and adipose tissues weights

Liver and adipose tissues weights were expressed as relative weight per body weight (Table 2). The relative weight of the liver had no significantly higher in the HFD group compared with other groups. The relative weight of the epididymal WAT and perirenal WAT were significantly lower in the ND, OR, PMC-1, or PMC-2 group than in the HFD group. Overall, the supplementation with PMC (orally) resulted in significantly lower weights of WAT compared to that of the HFD group.

Serum lipid levels

Concentrations of serum lipids are shown in Table 3. PMC and OR significantly lowered the serum Total-C concentration, TG concentration, LDL-C concentration, and the ratio of LDL-C/ Total-C compared to the HFD group. Supplementation of oral PMSBEC or OR exhibited a trend towards increased serum HDL-C levels, compared to HFD group, and the ratio of HDL-C/ Total-C exhibited higher values in the PMC-1, PMC-2 or OR group than in the HFD group.

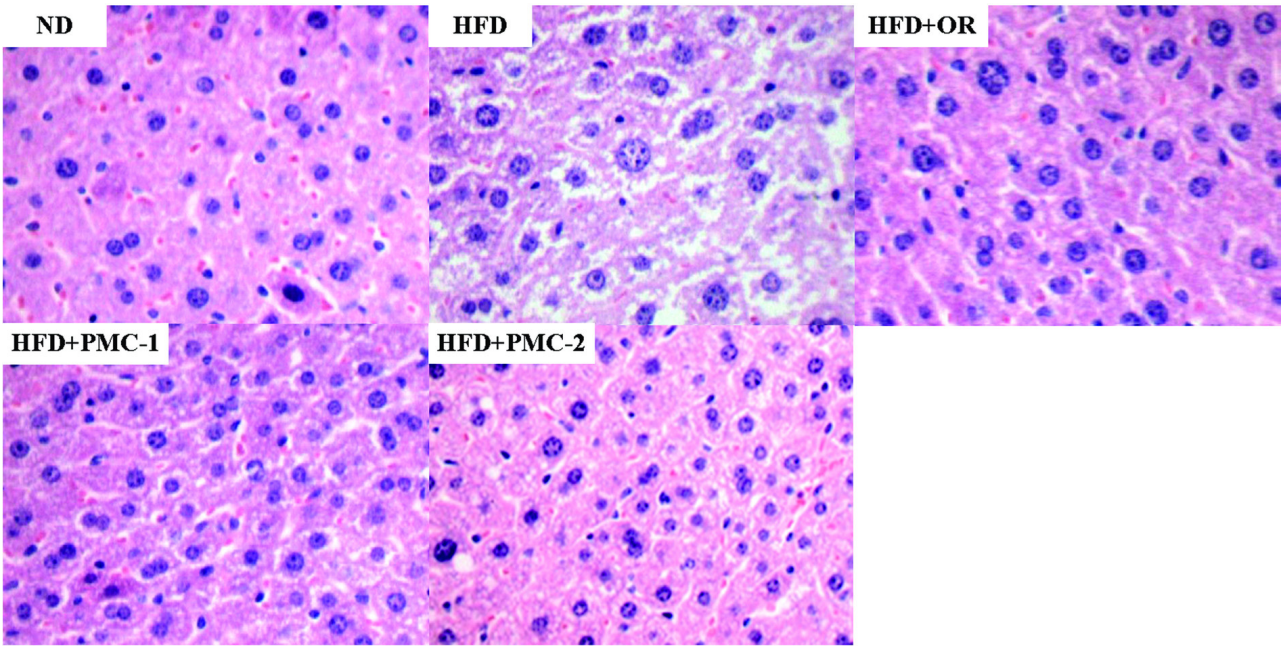

Figure 1. Effects of PMC on the liver tissue. Histological analysis of livers treated with HFD, HFD+OR, HFD+PMC-1 and HFD+PMC-2. Liver sections were stained with hematoxylin and eosin. Magnification ×400. See Table 1 for a description of the different experimental groups.

Effects of PMC on the liver

The livers in the HFD group were enlarged and produced a yellowish color, indicating liver steatosis. On the contrary, administration of PMC or OR reversed the liver to remain red and healthy. As shown in Figure 1, in histological
analysis, the liver of HFD-treated mice exhibited a typical sign of fatty liver showing the accumulation of many fat droplets and tissues through the liver acini. When mice were treated with PMC or OR, however, a much smaller degree of lipid accumulation and fewer pathological signs were observed.

DISCUSSION

P. mume has been traditionally used as food and used in folk remedies and some of its pharmacological actions have been partly confirmed by modern science (Adachi et al., 2007), and the explorations of *P. mume* products development have achieved enormous popularity (Ng et al., 2005). Although a high intake of polyphenols and flavonoids significantly reduced the risk of obesity and hyperlipidemia (Woo et al., 2008), the physiological roles of PMSBE combined with L-carnitine as anti-obesity and in improving lipid profile are not clear. Squalene synthase plays an important role in the cholesterol biosynthetic pathway. Chlorogenic acid screened from *P. mume* fruits could inhibit the squalene synthase of pig liver with an IC_{50} level of 100 nM (Choi et al., 2007). Our previous study indicated that chlorogenic acid was the principal phenolic compounds of PMSBE, followed by neochlorogenic acid and cryptochlorogenic acid.

The present study revealed that body weight gain of the HFD group mice were significantly higher than in the ND group, and the weight gain of PMC-1, PMC-2 and OR groups were normalized to the ND group (Table 1). In particular, the relative weight of the epididymal WAT and perirenal WAT of the HFD group mice were notably higher than any other group (ND, OR, PMC-1, or PMC-2) (Table 2). There were no abnormalities in growth performance; the relative weight of the liver had no significantly higher in the HFD group compared with other groups. Interestingly, feed intake of all experimental groups had no significantly different. High Total-C or LDL-cholesterol concentrations are a risk factor for coronary heart disease (Woo et al., 2008). High-fat diets significantly increase the Total-C levels in the serum and liver as compared with the normal control diet in mice (Ono et al., 2006). The clinical complications of atherosclerosis could be diminished when serum lipid concentration is lowered by hypocholesterolemic agents (Yang et al., 2006). The effects of polyphenols or flavonoids on lipid profile are very relevant to cardiovascular diseases. In this study, the average total phenolics content and the total flavonoids content of PMSB were 401.0 mg GAE/g and 355.5.5 mg RE/g, respectively. The anti-obesity functional formula (PMC) improved lipid profile by lowering serum Total-C, TG, LDL-C concentrations, and the ratio of LDL-C/ Total-C as compared with the HFD group (Table 3). The HDL-C concentration and the ratio of HDL-C/ Total-C significantly

increased compared with that of the HFD group. When mice were treated with PMC, a much smaller degree of lipid accumulation and fewer pathological signs were observed, as compared to the HFD group (Table 3). The PMC concentrations used in our study are proper concentrations to see the physiological benefits without any side effects.

Intestinal lipase inhibition using orlistat has been widely used in the pharmacotherapy of morbid obesity. However, the effects of orlistat on the secretion of appetite regulating gastrointestinal hormones and appetite sensations are still debated (Ellrichmann et al., 2008). The present study showed that PMC was comparable to orlistat in anti-obesity and improving lipid profile aspects of mice fed high-fat diet.

In conclusion, various functional components, such as flavonoids and polyphenols in PMSBE, could play important roles in altering body fat and regulating lipid metabolism. This study may have important implications because it is the first report that *P. mume* stem bark extract has anti-obesity and improving lipid profile effects. How- ever, further study is needed to clarify that the anti-obesity and hypolipidemic activity could be due to either PMSBE or L-carnitine or both, and its profound and subtle mechanisms.

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